IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Springer *et al.*

Appl. No. 09/220,227

Filed: December 23, 1998

For: **Analogs of Human Basic Fibroblast Growth Factor**

Art Unit: 1647

Examiner: C. Saoud -

Atty. Docket: 1503.0220001/JAG/THN

Declaration of Barry A. Springer, Ph.D. Under 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, Dr. Barry A. Springer, declare and state that:

1. I am one of the inventors of the above-captioned application. An accurate list of my credentials is set forth in my *Curriculum Vitae* attached as Appendix 1.

2. From 1994 to present, I have been employed by 3-Dimensional Pharmaceuticals, Inc. where I currently hold the position of Director of Molecular Biology & Protein Biochemistry. As part of my responsibilities at 3-Dimensional Pharmaceuticals, Inc., I supervised and/or conducted numerous experiments involving the effects of amino acid substitutions on protein function.

3. It is my understanding that the above-captioned application has claims pending directed to, e.g., muteins of human basic fibroblast growth factor (bFGF), or biologically active peptides thereof, that have improved mitogenic agonist activity and have substitutions of any

neutral and/or hydrophobic amino acids for one or more of the positions glutamate 89, aspartate 101 or leucine 137, where the numbering of amino acids is based on SEQ ID NO:1.

I have the following comments concerning the amount and type of guidance for substituting those positions with any neutral or hydrophobic amino acid that is provided in the specification and the literature at the time the above-captioned application was filed.

Amino Acid Positions Glutamate 89, Aspartate 101 and Leucine 137 Important to Mitogenic Agonist Activity

4. I and my co-inventors discovered that the amino acid positions glutamate 89, aspartate 101 and leucine 137, wherein the numbering of amino acids is based on SEQ ID NO:1, are important to the mitogenic agonist activity of bFGF. In particular, we discovered that the substitution of one or more of those amino acids with alanine, a neutral amino acid, results in a mutein which has improved mitogenic agonist activity over wild-type bFGF. Also, we discovered that the substitution of glutamate 89 with tyrosine, a hydrophobic amino acid, results in a mutein with similar improved mitogenic agonist activity.

Substitution with Alanine is Representative of Substitution with Any Neutral or Hydrophobic Amino Acid

5. The biological effect seen with the substitution of one or more of glutamate 89, aspartate 101 and leucine 137 with alanine is predictive that a substitution of one or more of those positions with another neutral amino acid and/or a hydrophobic amino acid will result in a mutein

with improved mitogenic agonist activity over wild-type bFGF. In other words, since it has been shown in the present application that alanine substitution at one or more of the above-mentioned positions produces a mutein with improved mitogenic agonist activity, it is reasonable to expect that when one or more of the above-mentioned positions is replaced with another neutral amino acid and/or a hydrophobic amino acid, the improved mitogenic agonist activity of the mutein will also be obtained. This is due to the fact that glutamate 89, aspartate 101 and leucine 137 are located on the surface of the human bFGF, and that neutral and/or hydrophobic substitutions on the surface of the protein are accommodated and do not affect the structure of the protein. See below ¶'s 6-10.

6. There is X-ray crystallographic evidence in the literature showing that glutamate 89, aspartate 101 and leucine 137 are located on the surface of human bFGF. See for example, Zhu *et al.*, *Science 251*:90-93 (1991) (Appendix 2); Zhang *et al.*, *Proc. Natl. Acad. Sci. USA 88*:3446-3450 (1991) (Appendix 3); and Erikson *et al.*, *Proc. Natl. Acad. Sci. USA 88*:3441-3445 (1991) (Appendix 4). Although the numbering system in the above documents varies depending on the exact form of the protein crystallized, in all cases the corresponding residues to glutamate 89, aspartate 101 and leucine 137 are well resolved and surface exposed.

7. Bowie *et al.* has examined amino acid substitutions within a protein and the likelihood that such mutations will affect biological activity (*Science 247*:1306-1310 (1990); Appendix 5). The authors reported that the choice of substitution at a given amino acid residue depends, in part, on the location of the residue within protein's three dimensional structure. As discussed by Bowie and coworkers,

> In general, only hydrophobic or neutral residues are tolerated at buried sites in proteins, undoubtedly because of the large favorable contribution of the hydrophobic effect to protein stability .
>
> * * *
>
> [but] most of the highly exposed positions tolerate a wide range of chemically different side chains, including hydrophilic and hydrophobic residues.

Thus, since hydrophobic or neutral amino acids are tolerated even at buried sites of the protein, it is reasonable to expect that neutral or hydrophobic amino acids other than alanine are also tolerated at positions glutamate 89, aspartate 101 and leucine 137 which are on the surface of the human bFGF. Further, it is reasonable to expect that the biological effect seen with the substitution with alanine will also be seen by substitutions with neutral or hydrophobic amino acids other than alanine. The biological effect seen with the substitution of glutamate 89 with tyrosine supports this.

8. There is ample evidence in the literature showing that usually small, minor local changes in a protein do not change the activity or the overall structure of the protein. For example, Watson *et al.* indicate that single amino acid substitutions usually do not alter enzyme activity (*Molecular Biology of the Gene* (1987), pages 226-227; Appendix 6). The authors reported that:

> The ability of a polypeptide chain to be enzymically active does not require an exactly specified amino acid sequence . . . . In fact, evidence now indicate that amino acid replacements in many parts of a polypeptide chain can occur without seriously modifying catalytic activity.

Similarly, according to Bowie *et al.*, "proteins are surprisingly tolerant of amino acid substitutions." In addition, the authors reiterated well-known rules concerning permissible amino acid substitutions within a protein based on the biochemical structure of amino acids:

> If a particular property of a side chain, such as charge or size, is important at a given position, only side chains that have the required property will be allowed. Conversely, if the chemical identity of the side chain is unimportant, then many different substitutions will be permitted.

Thus, it can be reasonably expected that the substitution of alanine at one or more of the positions glutamate 89, aspartate 101 and leucine 137 with another neutral amino acid and/or a hydrophobic amino acid will provide the biological effect seen by alanine substitutions at those positions.

9. Furthermore, Cunningham *et al.* has used alanine-scanning mutagenesis to identify specific side chains in human growth hormone (hGH) that modulate binding to the hGH receptor cloned from human liver (*Science 244*:1081-1085 (1989); Appendix 7). The binding of seven different conformationally sensitive monoclonal antibodies to hGH and various alanine mutants was determined. The results indicated that the overall folding of the mutant proteins was indistinguishable from that of the wild-type hGH. The authors reported that:

> These observations are consistent with crystallographic data showing that single amino acid substitutions in proteins generally cause only small and local structural perturbations apart from the altered side chain.

10. In summary, as described in paragraphs 4-9 above, one of ordinary skill in the art would have reasonably expected on the filing date that the biological effect that is seen from the

substitution of one or more of the positions glutamate 89, aspartate 101, or leucine 137 with alanine is predictive of substitution at those positions by any neutral or hydrophobic amino acid, and by any combination. Further, one of ordinary skill in the art would also have reasonably expected on the filing date that the biological effect that is seen from the substitution of the position glutamate 89 with tyrosine is predictive of substitution by any neutral or hydrophobic amino acid at that position. Furthermore, in my opinion, one skilled in the art could reasonably extrapolate from substitution with alanine at positions aspartate 101 and leucine 137 to substitution with tyrosine at those positions.

Further, at the time the above-captioned application was filed one of ordinary skill in the art would have reasonably expected that only routine experimentation would have been required to prepare muteins according to the invention that have improved mitogenic agonist activity. This is discussed in more detail below.

Guidance of Protein Mutagenesis and Screening the Mutein bFGF Mitogenicity

11. The muteins of human bFGF, or biologically active peptides thereof, according to the present invention can be prepared by simple mutagenesis. At the time the above-captioned application was filed, plenty of guidance on protein mutagenesis was available to protein biochemists of ordinary skill. For example, methods described at page 9, lines 11-16, and at page 10, lines 3-11, of the specification and in Example 1 can be used.

12. The improved mitogenic agonist activity of the bFGF mutant proteins according to the invention can be determined by simply comparing mutein and wild-type bFGF stimulation

of fibroblast growth. The method of screening is described in detail in Example 3 of the specification. A person of ordinary skill at the time the above-captioned application was filed would have been able without undue experimentation to follow the guidance of the specification and determine whether a mutein has improved mitogenic agonist activity.

Summary

13. It is my opinion that, based on the specification and the state of the art at the time the above-captioned application was filed,

1) the biological effect seen with the substitution of one or more of the positions glutamate 89, aspartate 101, or leucine 137 with alanine is predictive of substitution by any neutral or hydrophobic amino acid, and by any combination, at those positions;

2) the biological effect seen with the substitution of the position glutamate 89 with tyrosine is predictive of substitution by any neutral or hydrophobic amino acid at that position; and

3) the biological effect seen with the substitutions of one or more of the positions glutamate 89, aspartate 101, or leucine 137 with alanine and of the position glutamate 89 with tyrosine together is predictive of substitution at one or more of those positions by tyrosine, and by any combination with alanine.

As described in detail above, this is because, substitution of one or more of the key amino acid positions to mitogenic agonist activity, i.e., glutamate 89, aspartate 101 or leucine 137, of human bFGF with alanine and substitution of glutamate 89 of human bFGF with tyrosine result in muteins with improved mitogenic agonist activity, those amino acid positions are on the surface of the protein, and substitutions on the surface do not affect the structure of the protein. Further, only routine experimentation would have been required to generate muteins of bFGF according to the invention having improved mitogenic agonist activity.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Date: Oct 9 2002

Barry A. Springer, Ph.D.

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BARRY A. SPRINGER

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EDUCATION

Ph.D. University of Illinois, Department of Biochemistry, Urbana, Illinois, 1989
M.S. University of Illinois, Department of Biology, Urbana, Illinois, 1983
B.S. University of Illinois, Department of Microbiology, Urbana, Illinois, 1981

POSITIONS

Director of Molecular Biology & Protein Biochemistry. 3-Dimensional Pharmaceuticals Inc., 5/98 - present
Responsibilities:
Direct supervision of 4 Ph.D.'s and 5 M.S./B.S. associate scientists.
Leader of multi-disciplinary teams to meet corporate objectives.
G-Protein Coupled Receptor (GPCR) project team leader. The program focus is to determine an X-ray crystal structure of a GPCR and develop novel HTS assays for drug discovery.
Tyrosine Kinase Receptor project team leader. Program objectives are to discover novel, orally active, small molecule inhibitors of angiogenesis (by targeting both the kinase and extracellular domains) for the treatment of tumor growth and metastasis.
Responsible for cloning, expression, purification and characterization of enzyme and receptor targets to support high-throughput screening and X-ray crystallography.
Manage outside industrial contracts and academic collaborations.
Presentation of therapeutic and technical programs to Board of Directors, Scientific Advisory Board, Venture Capitalists and Corporate Partners.
Member of the New Target Selection Committee.
A founding member of 3-Dimensional Pharmaceuticals, Inc. scientific staff.
Write and serve as principal investigator for NIST-ATP and NIH grants.

Assistant Director of Molecular Biology. 3-Dimensional Pharmaceuticals Inc., 7/96 - 5/98.

Principal Research Scientist, 3-Dimensional Pharmaceuticals Inc., 8/94 - 7/96

Senior Research Scientist and **Research Scientist,** The DuPont Merck Pharmaceutical Company, Departments of Chemical and Physical Sciences and Inflammatory Disease Research. 1/91 - 8/94.
Responsibilities:
Member of the Angiogenesis Working Group and the Angiogenesis Steering Committee.
Cloning, expression, and purification of growth factors and receptors.
Biochemical and biophysical characterization of protein-protein and protein-ligand binding interactions.
Supervision of 1 Ph.D. scientist and 1 M.S. associate.

Helen Hay Whitney Post-doctoral Fellow, Department of Chemistry, University of California, Berkeley, CA, 12/89 - 12/90.

Graduate Student and Post-doctoral Research Associate, Department of Biochemistry, University of Illinois, Urbana, Illinois, 8/85 - 11/89.

Research Biochemist I and II, Diagnostics Division, Abbott Laboratories, Abbott Park, North Chicago, Illinois. *Focus*: Development of Immunoassays for infectious diseases, 6/83 - 8/85.

TECHNICAL SKILLS

Molecular and Cell Biology

RT-PCR and cDNA gene cloning, total gene synthesis.
Site-directed mutagenesis (cassette, single stranded, PCR).
High level protein expression in heterologous hosts (*E. coli*, baculovirus, yeast, mammalian).
Fibroblast and endothelial cell proliferation assays, Cell based receptor binding assays.

Protein Engineering

Extensive use of site-directed mutagenesis to understand protein structure-function relationships.
Site-directed mutagenesis using unnatural amino acids and semi-synthetic approaches.
Engineering proteins for structure determination (X-ray and NMR).
Coordination of the aforementioned skills in understanding the molecular interactions between proteins and other molecules to accelerate the design and discovery of novel pharmaceuticals.

Protein Chemistry and Biophysics

Protein purification (FPLC, standard LC, and affinity chromatography), protein refolding from *E. coli* inclusion bodies.
Quantitative measurements of protein-protein and protein-ligand interactions using radio-ligand binding, ELISA, Isothermal Titration Calorimetry, and BIAcore.

Molecular Modeling and Structural Biology

Proficient in the use of Biosym's molecular graphics programs Insight and Discover.
Knowledgeable in protein structure determination methods and applications in rational drug design strategies.

HONORS AND AWARDS

Promoted from Assistant Director to Director, 1998, 3-Dimensional Pharmaceuticals.
Promoted from Principal Research Scientist to Assistant Director, 1996, 3-Dimensional Pharmaceuticals.
Performance and Excellence Award, 1994, The DuPont Merck Pharmaceutical Company.
Promoted from Research Scientist to Senior Research Scientist, 1993, The DuPont Merck Pharmaceutical Company.
Commitment to Excellence Award, 1993, The DuPont Merck Pharmaceutical Company.
Awarded a post-doctorate slot based on internal competition, 1993, The DuPont Merck Pharmaceutical Company.
Commitment to Excellence Award, 1992, The DuPont Merck Pharmaceutical Company.
Awarded a 1990 Helen Hay Whitney post-doctoral fellowship.
Ph.D. thesis awarded by the Biochemistry Trust, University of Illinois, Urbana, Illinois, as the outstanding thesis of 1988-1989.
Recognized by Abbott Laboratories in 1985 as a contributing Chlamydiazyme team member who helped bring a diagnostic assay for the detection of Chlamydia in urethral swabs to market.
Promoted by Abbott laboratories from Biochemist I to Biochemist II in 1984.
Rated as an excellent Immunology teaching assistant in the Spring 1982 semester and an outstanding Immunology teaching assistant in the Fall semester 1982 and Spring semester 1983 as determined by the College Teaching Evaluation and Improvement Committee, University of Illinois, Urbana, Illinois.

OTHER ACCOMPLISHMENTS

Principal Investigator for a funded Advanced Technology Program Grant (ATP): "Crystallization and Structural Determination of G-Protein Coupled Receptors," Funding source: U.S. Department of Commerce, National Institutes of Standards and Technology, $1,988,000.

Principal Investigator for a funded, NIH Phase I Small Business Innovation Research Grant (SBIR): "Expression of G Protein-Coupled Receptors," Funding source: Cell Biology and Biophysics, Natl. Inst. General Medical Sciences, $159,002.

Co-authored a funded, NIH Phase I Small Business Innovation Research Grant (SBIR): "Four-Helix Analog of a G-Protein Coupled Receptor," Funding source: Cell Biology and Biophysics, Natl. Inst. General Medical Sciences, $100,000.

Co-authored a funded, NIH Phase II Small Business Innovation Research Grant (SBIR): "Four-Helix Analog of a G-Protein Coupled Receptor," Funding source: Cell Biology and Biophysics, Natl. Inst. General Medical Sciences, $998,000.

Principal Investigator for a funded, NIH Phase I Small Business Innovation Research Grant (SBIR): "Protein Engineering a Receptor Antagonist," Funding source: Growth Factors, Endocrinology and Aids Research, Natl. Inst. Diabet/Digest/Kidney Diseases, $78,049.

INVITED SYMPOSIA

Miami Bio/Technology Winter Symposium, Advances in Gene Technology: Protein Engineering and Structural Biology, February 4-9, 1995, Fort Lauderdale, FL. Session on Molecular Recognition: "Identification and Concerted Function of Two Receptor Binding Surfaces on Basic Fibroblast Growth Factor Required for Mitogenesis."

American Chemical Society Western Regional Meeting, Western Biotech Conference, October 18-21, 1995, San Diego, CA. Session on Protein-Protein Interactions: "Understanding Growth Factor-Receptor Interactions: Toward the Design of Small Molecule Mimetics."

International Business Communications Third Annual Conference on New Advances in Peptidomimetics & Small Molecule Design, March 6-8, 1996, Washington, D.C. Session on Signal Transduction in Medicinal Chemistry: "Dissecting the Energetics of Growth Factor-Receptor Interactions: Toward the Design of Small Molecule Mimetics."

International Business Communications Conference on Protein Structure and Function: Applications for Drug Discovery, December 9-10, 1997, Coronado, CA. Session on Receptors: "Mapping the Surfaces of bFGF Involved in Receptor and Heparin Interactions: Application to Small Molecule Drug Design."

INVITED LECTURES

State University of New York, Syracuse, NY, Dept. of Biochemistry, 1994.
Pennsylvania State University, State College, PA, Dept. of Chemistry, 1995.
Johns Hopkins University, Baltimore, MD, Dept. of Biology, 1996.

PATENTS

Pantoliano, M.W., Rhind, A.W. ,Salemme, F.R., Springer, B.A., Bone, R., Petrella, E.C. "Microplate Thermal Shift Assay and Apparatus for Ligand Development and Multi-variable Protein Chemistry Optimization." PCT/US97/08154, WO 97/42500.

PUBLICATIONS

Springer B.A. and Sligar S.G. (1987) "High-Level Expression of Sperm Whale Myoglobin in *Escherichia coli.*" Proc. Natl. Acad. Sci., *84*, 8961-8965.

Olson, J.S., Mathews, A.J., Rohlfs, R.J., Springer, B.A., Egeberg, K.D., Sligar, S.G., Tame, J., Renaud, J.-P. and Nagai, K. (1988) "The Role of the Distal Histidine in Myoglobin and Haemoglobin." Nature *336,* 265-266.

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Morikis, D., Champion, P.M., Springer, B.A. and Sligar S.G. (1989) "Resonance Raman Investigations of Site-Directed Mutants of Myoglobin: Effects of Distal Histidine Replacement." Biochemistry, *28*, 4791-4800.

Rohlfs, R.J., Mathews, A.J., Carver, T.E., Olson, J.S., Springer, B.A., Egeberg, K.D. and Sligar S.G. (1990) "The Effects of Amino Acid Substitution at Position E7 (Residue 64) on the Kinetics of Ligand Binding to Sperm Whale Myoglobin." J. Biol. Chem. *265,* 3168-3176.

Phillips, G.N. Jr., Arduini, R.W., Springer, B.A. and Sligar S.G. (1990) "Crystal Structure of Myoglobin From a Synthetic Gene." Proteins: Structure, Function, and Genetics *7*: 358-365.

Egeberg, K.D., Springer, B.A., Sligar, S.G., Carver, T.E., Rohlfs, R.J. and Olson J.S. (1990a) "The Role of Val68(E11) in Ligand Binding to Sperm Whale Myoglobin." J. Biol. Chem. *265,* 11788-11795.

Morikis, D., Champion, P.M., Springer, B.A., Egeberg, K.D. and Sligar S.G. (1990) "Resonance Raman Studies of Iron Spin and Axial Coordination in Distal Pocket Mutants of Ferric Myoglobin." J. Biol. Chem. *265*, 12143-12145.

Egeberg, K.D., Springer, B.A., Martinis, S.A., Sligar, S.G., Morikis, D. and Champion P.M. (1990b). "Alteration of Sperm Whale Myoglobin Heme Axial Ligation by Site-Directed Mutagenesis." Biochemistry *29,* 9783-9791.

Bellelli, A., Antonini, G., Brunori, M., Springer, B.A. and Sligar S.G. (1990) "Transient Spectroscopy of the Reaction of Cyanide with Ferrous Myoglobin." J. Biol. Chem. *265*, 18898-18901.

Carver, T.E., Rohlfs, R.J., Olson, J.S., Gibson, Q.H., Blackmore, R.S., Springer, B.A. and Sligar S.G. (1990) "Analysis of the Kinetic Barriers for Ligand Binding to Sperm Whale Myoglobin Using Site-directed Mutagenesis and Laser Photolysis Techniques." J. Biol. Chem. *265*, 20007-20020.

Bormett, R.W., Asher, S.A., Larkin, P.J., Gustafson, W.G., Ragunathan, N., Freedman, T.B., Nafie, L.A., Balasubramanian, S., Boxer, S.G., Yu, N.T., Gersonde, K., Noble, R.W., Springer, B.A. and Sligar S.G. (1992) "Selective Examination of Heme Protein Azide Ligand-Distal Globin Interactions by Vibrational Circular Dichroism." J. Am. Chem. Soc. *114*, 6864-6867.

Van Dyke, B.R., Bakan, D.A., Glover, K.A.M., Hegenauer, J.C., Saltman, P., Springer, B.A., and Sligar, S.G. (1992) "Site-Directed Mutagenesis of Histidine Residues Involved in Cu(II) Binding and Reduction by Sperm Whale Myoglobin." Proc. Natl. Acad. Sci., *89*, 8016-8019.

Braunstein, D., Chu, K., Egeberg, K.D., Frauenfelder, H., Morant, J.R., Nienhaus, G.U., Ormos, P., Sligar, S.G., Springer, B.A. and Young, R.D. (1993) "Ligand Binding to Heme proteins: III. FTIR Studies of His-E7 and Val-E11 Mutants of Carbonmonoxymyoglobin." Biophys. J., *65*, 2447-2454.

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Pantoliano, M.W., Horlick, R.A., Springer, B.A., Van Dyk, D.E., Tobrey, T., Wetmore, D.R., Lear, J.D., Nahapetian, A.T., Bradley, J.D. and Sisk W.P. (1994) "Multivalent Ligand-Receptor Binding Interactions in the Fibroblast Growth Factor System Induce an Allosteric Growth Factor and Heparin Concerted Bridge Mechanism for Receptor Dimerization." Biochemistry, *33*, 10229-10248.

Springer, B.A., Pantoliano, M.W., Barbera, F.A., Gunyuzlu, P.L., Thompson, L.D., Herblin, W.F., Rosenfeld, S.A. and Book, G.W. (1994) "Identification and Concerted Function of Two Receptor Binding Surfaces on Basic Fibroblast Growth Factor Required for Mitogenesis." J. Biol. Chem., *269*, 26879-26884.

REVIEWS AND BOOK CHAPTERS

Stayton, P.S., Atkins, W.A., Springer, B.A. and S.G. Sligar (1989) "Site-Directed Mutagenesis of Heme Proteins," in *Metal Ions in Biological Systems*, volume 25: Interrelations Between Metal Ions, Enzymes, and Gene Expression (H. Sigel, ed.) Marcel and Dekker, New York.

Springer, B.A., Sligar, S.G., Olson J.S. and Phillips Jr., G.N. (1994) "Mechanisms of Ligand Recognition in Myoglobin" Chemical Reviews, *94*, 699-714.

ABSTRACTS

Sligar, S.G., Springer, B.A., Egeberg, K.D., Braunstein, D., Frauenfelder, H., Hong, M.-K. and Ormos, P. (1988) "FTIR Studies of Genetically Engineered Sperm Whale Myoglobin." Biophys. J. *53*, 247a.

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Springer, B.A., Egeberg, K.D., Sligar, S.G., Rohlfs, R.J., Mathews, A.J. and Olson, J.S. (1988) "Site-Directed Mutagenesis of Sperm Whale Myoglobin: Role the HisE7 and ValE11 in Ligand Binding." Symposium on Oxygen Binding Heme Proteins: Structure, Dynamics, Function and Genetics, Asilomar Conference Grounds, Pacific Grove, California October 9-13.

Egeberg, K.D., Springer, B.A., Sligar, S.G., Morikis, D. and Champion, P.M. (1988) "Alteration of Sperm Whale Myoglobin Axial Ligation by Site-Directed Mutagenesis." *ibid.*

Sligar, S.G., Springer, B.A., Egeberg, K.D., Ropp, J. and Chiu, M. (1988) "Mechanisms of Molecular Recognition in Heme Proteins: Structure, Dynamics, Function, and Genetics." *ibid.*

Morikis, D., Champion, P.M., Springer, B.A. and Sligar S.G. (1988) "Resonance Raman Studies of Site-Directed Mutants and Single Crystals of Myoglobin." *ibid.*

Braunstein, D., Egeberg, K.D., Frauenfelder, H., Mourant, J., Ormos, P., Sligar, S.G., Springer, B.A. and Young R.D. (1988) "FTIR Studies of CO Orientation in HisE7 Mutants of Carbonmonoxymyoglobin." *ibid.*

Olson, J.S., Carver, T.E., Rohlfs, R.J., Gibson, Q.H., Springer, B.A., Egeberg, K.D. and Sligar S.G. (1988) "Dynamics of Ligand Binding to Myoglobin: Quantum Yields, Geminate Recombination, Isonitriles, and E7 Mutants." *ibid.*

Sligar, S.G., Springer, B.A., Stayton, P.S., Atkins, W.A. Rodgers, K., Davies, M., Ropp, J. and Pochapsky T. (1988) "Site Directed Mutagenesis of Heme Proteins." American Chemical Society National Meeting, Los Angeles, September 27.

Braunstein, D., Cowen, B.R., Egeberg, K.D., Frauenfelder, H., Mourant, J., Ormos, P., Sligar, S.G., Springer, B.A. and Young R.D. (1989) "FTIR Studies of CO Orientation and pH Effects in HisE7 Mutants of Carbonmonoxymyoglobin." Biophys. J. *55*, 565a.

Sligar, S.G., Springer, B.A., Stayton, P.S., Rodgers, K. and Nagamune T. (1989) " Mechanisms of Macromolecular Recognition as Probed by Site-Directed Mutagenesis." The 1989 International Chemical Congress of Pacific Basin Societies.

Brunori, M., Antonini, G., Bellilli, A., Springer, B.A., and Sligar S.G. (1990) "Probing the Stereochemistry of the Distal Site of Hemoglobin and Myoglobin with Cyanide, the Only Charged Ligand of Ferrous Heme Iron." The Dynamics and Kinetics of Myoglobin and Hemoglobin, Bethesda, MD, June 25-27.

Olson, J.S., Springer, B.A., Egeberg, K.D., Sligar, S.G., Smerdon, S., Wilkinson, A.J., Dodson, G.G., Gibson, Q.H., Carver, T.E. and Rohlfs R.J. (1990) "Analysis of the Kinetic Barriers for Ligand Binding to Myoglobin." *ibid.*

Cutruzzola, F., Brunori, M., Springer, B.A. and Sligar, S.G. (1990) "Synthesis of *Aplysia Limacina* Myoglobin Gene." 10th International Biophysics Congress, Vancouver Canada.

Asher, S., Larkin, P., Ragunathan, N., Freedman, T., Nafie, L., Springer, B.A., Sligar S.G. and Noble, R. (1990) "Vibrational Circular Dichroism, Raman FTIR, and Electronic CD Studies of Azide Binding to Heme Proteins." Biophys J. *57*, 233.

Asher, S.A., Bormett, R.W., Larkin, P.J., Gustafson, W.G., Ragunathan, N. Freedman, T.B., Nafie, L.A., Yu, N.-T., Gersonde, K., Noble, R.W., Springer, B.A. and Sligar S.G. (1991) in *Spectroscopy of Biological Molecules* (R.E. Hester and R.B. Girling, eds.) Royal Society of Chemistry, Cambridge, England.

Thompson, L.D., Springer, B.A., Pantoliano, M.W. and Van Dyk D.E. (1993) "Identification and Characterization of the Heparin Binding Sites on Basic Fibroblast Growth Factor." American Society for Biochemistry and Molecular Biology/Division of Biological Chemistry-American Chemical Society Joint Meeting, May 1993, San Diego, CA. FASEB J., Vol. 7, No. 7 (APR 20), p 1279.

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